Available online at www.sciencedirect.com
ScienceDirect
Regulatory Peptides 137 (2006) 162–167
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

A new technique for in vivo imaging of specific GLP-1 binding sites: First results in small rodents

Martin Gotthardt [a,*], Georg Lalyko [b], Julliëtte van Eerd-Vismale [a], Boris Keil [c], Tino Schurrat [b], Michael Hower [b], Peter Laverman [a], Thomas M. Behr [b], Otto C Boerman [a], Burkhard Göke [d], Martin Béhé [b]

[a] Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, Postbus 9101, 6500 HB Nijmegen, The Netherlands
[b] Department of Nuclear Medicine, Philipps-University of Marburg, Baldingerstrasse, 35043 Marburg, Germany
[c] Department of Diagnostic Radiology, Philipps-University of Marburg, Baldingerstrasse, 35043 Marburg, Germany
[d] Department of Internal Medicine, Ludwig-Maximilians-University of Munich, Marcionistrasse 15, 81377 München, Germany

Received 19 April 2006; received in revised form 13 July 2006; accepted 13 July 2006
Available online 22 August 2006

Abstract

Experimental objectives: In vivo imaging of GLP-1 receptor-positive tissues may allow examination of physiologic and pathophysiologic processes. Based on the GLP-1 analog Exendin 4, we have developed a radiolabeled compound specifically targeting the GLP-1 receptor (DTPA-Lys_{40}-Exendin 4). This work aims to detect GLP-1 receptor-positive tissues by biodistribution studies and in vivo small animal imaging studies. For in vivo imaging, a high-resolution multi-pinhole SPECT (single photon emission computed tomography) system was used in conjunction with an MRI (magnetic resonance imaging) system for image fusion.
Results: DTPA-Lys_{40}-Exendin 4 can be labeled with ^{111}In to high specific activity (40 GBq/μmol). The radiochemical purity reliably exceeded 95%. Using this compound for in vivo small animal imaging of rats and mice as well as for biodistribution studies, specific GLP-1 binding sites could be detected in stomach, pancreas, lung, adrenals, and pituitary. Receptor-positive tissues were visualized with a high-resolution SPECT system with a resolution of less than 1 mm.
Conclusions: The new technique using DTPA-Lys_{40}-Exendin 4 allows highly sensitive imaging of GLP-1 receptor-positive tissues in vivo. Therefore, intra-individual follow-up studies of GLP-1 receptor-positive tissue could be conducted in vivo.

Keywords: GLP-1; Receptor imaging; Small animal imaging; Single photon emission computed tomography; Magnetic resonance imaging; Exendin 4

1. Introduction

Glucagon-like Peptide-1 (GLP-1) plays an important role in glucose metabolism and homeostasis. GLP-1 is secreted after ingestion of food and induces insulin-release from the pancreas in a blood-glucose level-dependent manner. Due to its glucose-lowering action, GLP-1 or analogs thereof may be used to treat diabetes mellitus type 2 [1]. The insulinotropic action of GLP-1 is mediated by a specific receptor on the β-cells in the islets of Langerhans in the endocrine pancreas [2]. Besides its direct insulinotropic action, GLP-1 also has effects on glucagon secretion and reduces gastric motility, both factors (indirectly) influencing the blood-glucose level [1,3]. Furthermore, it induces proliferation of β-cells [4]. Despite its effects on the pancreas, GLP-1 also seems to play a role in other organ systems. Intracerebroventricular injection of GLP-1 can reduce food intake in rats [5]. Though GLP-1-binding sites are widespread in the brain [6], peptidic hormones do not cross the blood–brain barrier due to their hydrophilicity [7]. The effects of GLP-1 on satiety and food intake may thus be secondary to decelerated gastric emptying [8] or may be mediated by binding to sites in the brain that are not protected by the blood–brain barrier, such as the area postrema in the brain stem [9,10]. Besides in pancreas, stomach [11], and brain, GLP-1 receptor

* Corresponding author. Tel./fax: +31 24 36 15054/18942.
E-mail address: m.gotthardt@nucmed.umcn.nl (M. Gotthardt).

0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.07.005

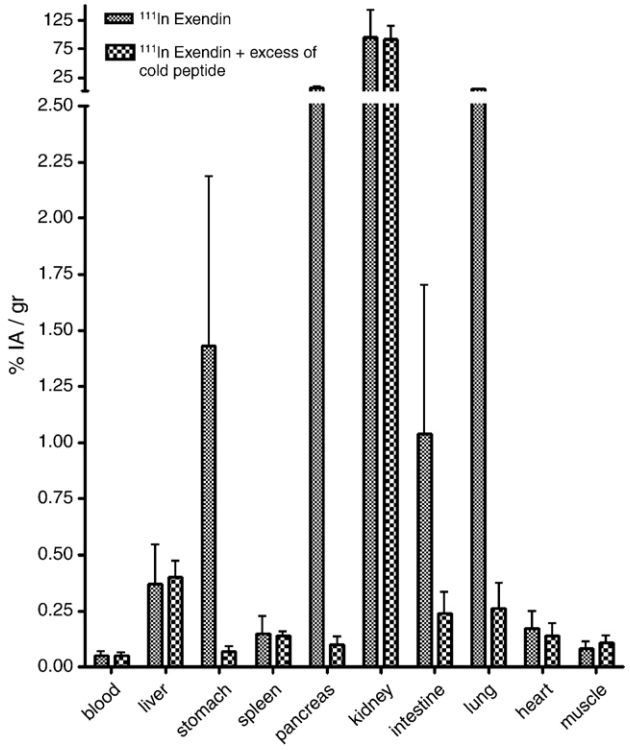

Fig. 1. Biodistribution of 1 MBq ^{111}In-DTPA-Lys^{40}-Exendin 4 alone and blocked by co-injection of 100 μg unlabeled Exendin 4 20 h after injection in mice. Specific uptake in lung, pancreas, and stomach. Due to the large standard deviation, intestinal uptake does not differ significantly.

expression has also been shown on mucous-producing cells of the bronchial mucosa and in the smooth muscle cells of the pulmonary arteries in rat lungs [12,13]. GLP-1 receptor mRNA has been identified in the kidney, duodenum, and heart [14,15]. In liver, skeletal muscle, and adipose tissue, GLP-1 receptor mRNA has not been shown [14]. In contrast to these findings, other studies did not demonstrate specific binding sites in the stomach [16] or indicate that GLP-1 has specific effects on adipose tissue depending on the presence of a receptor [17–19]. Furthermore, studies evaluating distribution of the GLP-1 receptor or the specific uptake in vivo have been done with radioiodinated compounds which are known to show a relatively low uptake as the radionuclide is quickly released from tissue after receptor-mediated uptake of the ligand [19–22].

A technique would therefore be desirable for non-invasive examination of the GLP-1 receptor distribution in vivo. This technique could also allow the detection of tumors expressing the GLP-1 receptor, such as carcinoids [23,24]. Using a modified Exendin 4, we have developed a new GLP-1 receptor-targeting compound for scintigraphic tumor imaging [23]. Exendin 4 is a GLP-1 analog that has been found in the venom of Gilamonsters [25]. Exenatide, which is synthetic Exendin 4, has currently been approved (US) or will be approved in the near future (Europe) for treatment of diabetes type 2 [1,4]. It is metabolically much more stable in comparison to GLP-1, has comparable actions, and shares a 53% homology [25,26]. Due to its higher stability, it is more suitable for radiopeptide imaging or therapy than GLP-1. This compound has therefore been labeled with a so-called residualizing label which is retained in the cell after internalization to improve the target-to-background ratios [22,27,28]. In this communication, we show the first results obtained with the new compound. Besides acquiring biodistribution data, high-resolution small animal imaging has been performed.

2. Material and methods

2.1. Tracer labeling

Labeling of the DTPA-conjugated Lys^{40}-Exendin 4 was done essentially as previously described [29]. In short, ~40 MBq $^{111}InCl_3$ in 100 μL 0.05 M HCl were added to 100 μL of the peptide solved at a concentration of 50 μM in ammonium acetate buffer, pH 5.0. After 30 min, quality control was performed using high performance liquid chromatography (HPLC) on a C-18 reverse phase column. Two buffers were used as mobile phase (buffer A 70% actonitrile, H_2O, 0.1% TFA, pH 7.4; buffer B H_2O, 0.1% TFA, pH 7.4) with a linear gradient rising to 70% acetonitrile (100% buffer A) over 30 min.

2.2. Animals, biodistribution studies

Male Wistar rats weighing 250 g were obtained at an age of 8 weeks from Harlan (Horst, The Netherlands). Nude mice (BALBc *nu*/*nu*) obtained from Charles River Wiga (Sulzfeld, Germany), were 8 weeks old and weighed 25 g. The animal experiments had been approved by the local animal welfare committees in Nijmegen and Marburg.

For biodistribution experiments 1 MBq ^{111}In-DTPA-Lys^{40}-Exendin 4 was intravenously injected via the tail vein. Four animals were used per group. The respective control groups were

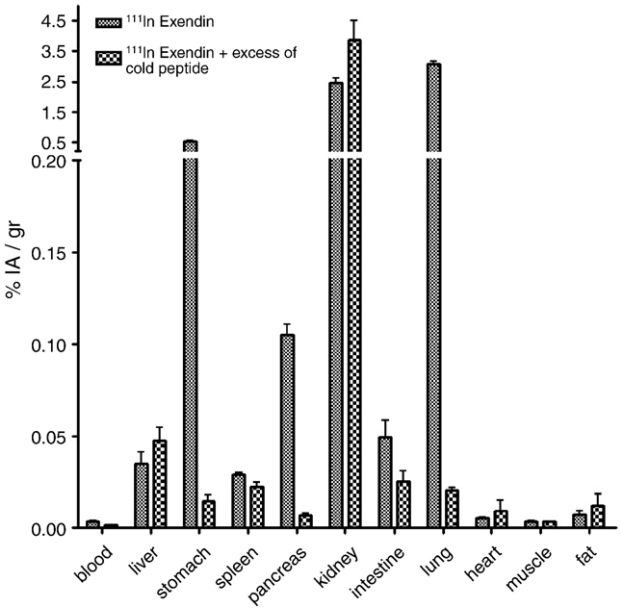

Fig. 2. Biodistribution of 1 MBq ^{111}In-DTPA-Lys^{40}-Exendin 4 alone and blocked by co-injection of 100 μg unlabeled Exendin 4 20 h after injection in rats. Specific uptake in lung, pancreas, spleen, and stomach. Due to the large standard deviation, intestinal uptake does not differ significantly.